UNIVERSITY OF ILLINOIS
AT URBANA-CHAMPAIGN

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May 4, 1999

Dockets Management Branch (HFA-305)
Food & Drug Administration
5630 Fishers Lane, rm. 1061
Rockville, MD 20852

Dear Dockets Management Branch:

I am writing in response to your solicitation for information on sunlamp products.

Recently, we completed a study of the effects of UV-A radiation (the dominant UV rays of most tanning lamps) upon the epidermal ultraviolet chromophore *trans*-urocanic acid (*t*-UA). *t*-UA is the dominant UV absorbing molecule in the uppermost layer of the skin (stratum corneum) and has been implicated to behave as a mediator of immune suppression following UV-B absorption. Our research showed that in the UV-A, *t*-UA sensitized the production of reactive oxygen species like singlet oxygen, which are believed to lead to the premature aging and structural degrádation of the skin.

I have enclosed a copy of the original paper discussing this work. I've also enclosed articles that resulted from a NIH press-release written in September of 1998 on this work. These give a general description of the effects of UV-A radiation upon the skin, but do not specifically mention the source of UV-A radiation to be from tanning beds. The American Academy of Dermatology (AAD) in October of 1998, however, subsequently wrote a press-release for their National Healthy Skin Program that related our *t*-UA-UV-A research to the effects of tanning lamps upon the skin. I've included a copy of the AAD press-release for you, as well.

If you have any questions on the information I have enclosed please do not hesitate to contact me.

Sincerely,

Kerry Hanson

98N-1170

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Date: Tue, 04 May 1999 08:41:16 -0400
From: Sun Protection Listserver <ROSSEEL.KEVIN@epamail.epa.gov> (KEVIN ROSSEEL)
Subject: FDA and sunlamp labeling
To: sunprotection@Mailing-List.net
X-Mailer: Novell GroupWise 4.1
Sender: owner-sunprotection@Mailing-List.net

The Food and Drug Administration (FDA) is soliciting comments and information from interested persons concerning the following:

1) adequacy of warnings on sunlamp products,
2) current recommended exposure schedule to minimize risk to customers who choose to produce and maintain a tan,
3) current labeling for replacement lamps, and
4) current health warnings which do not reflect recent advances in photobiological research.

Written comments are due by May 10, 1999.

ADDRESSES: Submit written comments to the Dockets Management Branch (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. Individuals or organizations wishing to receive copies of draft amendments or related documents distributed for review during the development of these amendments may have their names placed on a mailing list by writing to Office of Science and Technology (HFZ-114), Center for Devices and Radiological Health, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, FAX 301-594-6775, e-mail address HWC@CDRH.FDA.GOV.

FOR FURTHER INFORMATION CONTACT: W. Howard Cyr, Center for Devices and Radiological Health (HFZ-114), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, 301-443-7179.

Full text of the Advanced Notice of Proposed Rulemaking notice is available from the Federal Register website at:

http://frwebgate.access.gpo.gov/cgi-bin/waisgate.cgi?WAISdocID=212238583+0+0+0&WAISaction=retrieve

MARTIN PARR – MAGNUM

**Grab the sunscreen: It's not the years, but the amount of time in the sun that damages skin.**

# Deep-fried by the sun

## Ultraviolet-A proves to be the culprit in causing the wrinkles and sags of age

BY MARISSA MELTON

Scientists have long suspected that ultraviolet-A rays, which make up 95 percent of the sunlight we encounter each day, damage skin, causing wrinkling, spotting, and leatheriness over time. But all they could prove was that UV-B rays, the kind blocked by most sunscreens, cause sunburn and cancer. Now, researchers do have undeniable evidence showing that UV-A is dangerous because of its effect on a substance once believed to be a "natural sunscreen."

Chemists John Simon of Duke University and Kerry Hanson of the University of Illinois–Urbana-Champaign knew that transurocanic acid (trans-UA), an amino acid product found in the top layer of skin, takes in UV-B rays and changes its shape. But when they tried to account for the energy used in that change, they found trans-UA was absorbing almost three times as much energy as needed. That extra energy consumption meant something else was happening.

Using a process called photoacoustic spectroscopy, they aimed an ultraviolet laser at trans-UA molecules to excite them into activity. They then measured the amount of heat the molecules released as the change occurred. They found that when trans-UA is hit by UV-A rays at the lower end of the spectrum, some of the energy from the trans-UA is used to create oxygen free radicals, chemical rogues that damage and prematurely age skin. Oxygen free radicals also damage other parts of the body, including the respiratory and immune systems.

**No cancer link.** Although the chemists' work provides the first molecular evidence that UV-A rays harm the skin, it does not, Simon cautions, show a direct link between UV-A and skin cancer, as has been proved with UV-B, although scientists suspect UV-A plays a role, too.

Unfortunately, few sunscreens on the market protect the skin from UV-A rays, and the "SPF" (sun-protection factor) scale by which a sunscreen's strength is measured applies only to UV-B radiation. Sunblocks with zinc oxide and titanium dioxide and sunscreens with the ingredient avobenzone provide the best UV-A protection; check labels before you buy. Need incentive? Up to 90 percent of the skin deterioration we attribute to chronological aging is actually induced by ultraviolet A and B. ■

# It's a fat, fat world

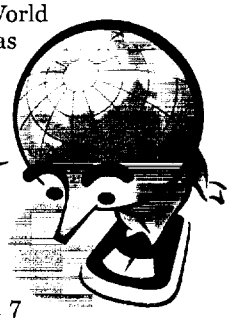
## Prosperity blamed for health threat

BY BRENDAN I. KOERNER

Americans are used to being admonished for their self-destructive fondness for cheese-flavored snacks and La-Z-Boys. But citizens of developing countries are also falling prey to the perils of the First World's fat-rich diet and couch-potato ways. At last week's 8th International Congress on Obesity, held in Paris, researchers warned that the planet's expanding waistlines threaten "to become the curse of the next millennium."

"We used to consider obesity a problem of industrialized, rich countries," says Arnaud Basdevant, a member of the meeting's lead committee. "But now it has become a world pandemic."

Researchers blame the trend—and the attendant rise in the incidence of heart disease and diabetes—on the Third World's increasing affluence, reduced physical activity, and more calorie-laden diets. As a result, the World Health Organization has estimated that 300 million people will be obese by 2025, an increase of 50 million from today. In Mauritius, for example, WHO estimates that 32 percent of the population will be obese by 2025, compared with 7 percent in 1987. One in five Brazilians are expected to be obese in 2025, more than double the number in 1989. Last year, WHO said that obesity's lethal impact could rival smoking.



There was much hopeful discussion about new drugs such as leptin, a hormone that has proved effective for severe weight problems. But leptin should be a last resort, says Basdevant: "First of all, we have to consider prevention." If America is any indication, however, educating the world about the perils of Big Macs and cable TV will be difficult: Despite heightened awareness of the need for proper diet and exercise, the number of obese Americans is expected to double over the next three decades. ■

ILLUSTRATION BY STEVE MCCRACKEN FOR *USN&WR*

NIH NEWS RELEASE

NATIONAL INSTITUTES OF HEALTH

National Institute of General Medical Sciences

EMBARGOED BY JOURNAL
5:00 pm ET August 31, 1998

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**UV Skin Damage in a Different Light**

Despite all the warnings to avoid exposure to the sun and to wear sunscreen, scientists don't really know how the sun damages our skin. Now, they're a bit closer to the answer.

Two scientists recently discovered that sunlight triggers a harmful reaction when it strikes a molecule in our skin--ironically a molecule once thought to be "nature's sunscreen." The work suggests the science behind the sagging, leathery skin typical of long-term sun worshipers, and may also shed light on how ultraviolet light causes skin cancer.

"We studied a natural component of human skin exposed to ultraviolet light and uncovered a new chemical reaction that may contribute to aging [of the skin] and cancer," said Dr. John Simon, who led the study.

The research will appear in the September 1 issue of the *Proceedings of the National Academy of Sciences*. The work was conducted at the University of California, San Diego by Dr. John D. Simon (who is now at Duke University), and his graduate student Kerry Hanson (who is now a postdoctoral fellow at the University of Illinois at Urbana-Champaign).

The sun's harmful rays come in two flavors: ultraviolet A and ultraviolet B. Evidence mounts that ultraviolet A and B both play a role in causing skin cancer and photoaging, which is characterized by deep, premature wrinkles, thickened skin, and age spots.

Time, gravity, and heredity notwithstanding, "something like 90 percent of all the visible signs of aging are from ultraviolet sources," said Dr. Kerry Hanson. "Photoaging is not just a cosmetic effect. It destroys the integrity of your skin."

The focus of this study is the sun-sensitive molecule called *trans*-urocanic acid (*t*-UA). Formed in the top layer of the skin, *t*-UA molecules cover our bodies, acting like antennae for light. In the 1950s, urocanic acid was hailed as a "natural sunscreen" because it absorbs ultraviolet B light. It was thus thought to protect against damage by such rays, which can potentially lead to skin cancer. For a time, the pigment was even added to sunscreens and skin lotions.

When exposed to ultraviolet rays, *trans*-UA buckles in upon itself to form *cis*-UA. Most theories about urocanic acid's action focus on this structural flip and subsequent chemical reactions. But the data didn't seem to fit--urocanic acid was absorbing almost three times more energy than could be accounted for by its twist into its *cis* form. It must undergo some additional chemical reaction, the scientists reasoned. So they investigated the molecule in a different light--literally. What they found revealed not only the molecule's additional maneuver, but its sinister consequences.

Using a cutting-edge technique called photoacoustic spectroscopy, Drs. Hanson and Simon studied urocanic acid's activity when exposed to light near the tail end of the ultraviolet A range, where the molecule's reactivity was thought to be harmless. They discovered that when this type of light strikes *t*-UA, it zaps the molecule into an excited "triplet" state that sparks the creation of oxygen radicals.

(More)

Science

## Absorption of UV-A by chromophore may have role in photoaging of skin

By Faith Reidenbach

WESTPORT, Sep 01 (Reuters) - Trans-urocanic acid, a chromophore that forms in the epidermis, sensitizes reactive oxygen species when exposed to the tail end of ultraviolet A radiation (UV-A), according to report in the September 1st issue of the Proceedings of the National Academy of Science.

Dr. Kerry M. Hanson, now at the University of Illinois at Urbana-Champaign, and Dr. John D. Simon, now at Duke University, Durham, North Carolina, used pulsed laser photoacoustic spectroscopy to study how trans-urocanic acid is affected by UV-A between 310 and 351 nm. This instrumentation determines the energy of a molecule by trapping its heat, Dr. Hanson told Reuters Health.

From research by others, Drs. Hanson and Simon knew that trans-urocanic acid isomerizes into cis-urocanic acid under exposure to UV-A in the range of 310 to 351 nm. But they found that "...the energy retained after excitation at 340 nm is almost three times the energy difference between the two isomers."

"Consequently, we can conclude that photoisomerization is not the sole photochemical process that is initiated by UV-A exposure," the researchers write. "To account for this observed energy storage, a long-lived intermediate must be formed."

In further experiments, Drs. Hanson and Simon determined that the intermediate is an excited triplet state of trans-urocanic acid that can produce reactive oxygen species. They propose that the generation of these reactive oxygen species "...initiates chemical processes that lead to the physiological responses characteristic of UV-A photoaged skin."

"We don't know from our data specifically that urocanic acid leads to photoaging," Dr. Hanson told Reuters Health. "It's an indirect link right now. We do know that urocanic acid releases reactive oxygen species, specifically singlet oxygen. That is known to lead to photoaging effects within the skin, and also potentially to lead to immune suppression and skin cancer."

"Previously, people didn't think the higher wavelengths of UV-A radiation, like around 350 nanometers, had any adverse effects," Dr. Hanson added. "But our data, and other people's work, is showing that there is the potential for UV-A damage. All of us who spend time in the sun should wear a sunscreen that covers not only the UV-B range, but also the UV-A."

Proc Natl Acad Sci USA 1998;95:10576-10578.

-Westport Newsroom 203 319 2700

Oxygen radicals are chemical rogues blamed not only for premature aging, but also for damaging DNA, suppressing the immune system, and causing some respiratory problems.

"The results certainly surprised us," said Dr. Simon. "We never expected to see [any] process that allowed oxygen to get sensitized" because the study was conducted under ultraviolet light much different than that which most excites *t*-UA. "It's a real serendipity case."

"What it means is that you have to be more concerned about protecting yourself from ultraviolet A radiation," he continued. "We should probably use sunscreens that block all the way out to 400 nm [the end of the ultraviolet A region]."

The SPF (solar or sun protection factor) in sunscreen refers to its ability to protect against the burning rays of ultraviolet B light. But currently there are no world-wide standards to measure protection against ultraviolet A, which accounts for 95 percent of sunlight that reaches the earth.

The work may also change the way researchers approach similar projects. Instead of studying a biological molecule exclusively under the type of light that it absorbs most, Dr. Simon suggested that scientists may need to expose the molecule to a whole spectrum of light, slice by slice, to fully understand the molecule's physiological effects.

# # #

Please mention support for this work from the National Institute of General Medical Sciences (NIGMS), a component of the National Institutes of Health that supports basic biomedical research. Please fax clips to (301) 402-0224. After the embargo date, this release will be available on-line at http://www.nih.gov/nigms/news/releases/simon.html.

Reference
Hanson K, Simon J. Epidermal trans-Urocanic Acid and the UV-A Induced Photoaging of the Skin. *Proc. Natl. Acad. Sci. USA* 1998;95(18):10576-10578.

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For a copy of the paper during the embargo, journalists may call the National Academy of Sciences at (202) 334-2138.
For scientific perspective on this research, call the NIGMS Public Information Office at (301) 496-7301 to interview **Dr. Janna Wehrle**, program director, Division of Cell Biology and Biophysics.

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## RESEARCH LINKS UVA LIGHT TO SKIN DAMAGE

**SCHAUMBURG, IL (October 28, 1998)** -- Ultraviolet A (UVA) light, the type of light used in tanning beds, causes skin photoaging which can lead to wrinkles, age spots and skin cancer according to new research. Scientists discovered that when human skin is exposed to UVA light, it creates the oxygen radicals that are responsible for premature photoaging of skin, as well as damaging DNA, suppressing the immune system and causing some respiratory problems.

The research, which appeared in the September 1998 issue of *Proceedings of the National Academy of Sciences*, was conducted by John D. Simon, Ph.D., and Kerry M. Hanson, Ph.D.

The focus of the study was trans-urocanic acid (t-UA), a molecule found in the top layer of the skin. At one time, t-UA was considered to be a natural sunscreen because it absorbs ultraviolet B (UVB) light. Drs. Simon and Hanson used a cutting-edge technique called photoacoustic spectroscopy to discover that t-UA creates oxygen radicals when exposed to UVA.

-MORE-

"The wavelength range of the UVA that Drs. Simon and Hanson used in their research (320-400 nanometer wavelengths) is two to three times the amount of UVA in normal sunlight, but typical of the UVA radiation in tanning beds," said Rex Amonette, M.D. of the University of Tennessee in Memphis and past president of the American Academy of Dermatology. "For many years we have seen the negative effects of tanning, especially tanning beds, on the skin of our patients. Now we have scientific evidence that the radiation in tanning bed lights leads to the type of skin damage that causes skin cancer."

Dr. Amonette's research on tanning beds found that UVA light has short-term side effects that range from skin redness to itching to dry skin to nausea. Long-term effects include the development of skin cancers, photoaging, rashes from the interaction of the UVA and common medications, or even cause unusual diseases like polymorphic light eruption.

Indoor tanning involves bombardment of the skin with UVA light. Tanning beds are often advertised as a safe alternative to tanning under the sun because of the lack of UVB radiation. Scientists now understand that UVA is as responsible as UVB in photoaging of the skin and many other conditions. UVA has a longer wavelength than UVB, penetrating deeper into the skin to cause damage to its elastic fibers.

Natural skin aging is commonly characterized by a thinning of the skin and a deepening of the normal facial expression lines. Photoaging is distinguished by coarse wrinkles, inelasticity and discoloring. These changes, related to long-term exposure to the sun, are considered

indications for a person's risk of developing skin cancer. While more than 50% of a person's lifetime exposure to ultraviolet light generally occurs before age 18, it may take decades before the visible signs to photodamage appear.

The American Academy of Dermatology recommends everyone take daily steps to limit sun damage including:

1. Wear protective clothing, including hats
2. Choose a broad spectrum sunscreen (which protects against both UVA and UVB rays) with a sun protection factor (SPF) of at least 15 for daily use
3. Limit outdoor activities between 10 a.m. and 4 p.m. daily

The AAD is the largest medical society representing physicians who specialize in treating conditions of the skin, hair and nails.

# # #

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Biophysics

# Epidermal *trans*-urocanic acid and the UV-A-induced photoaging of the skin

KERRY M. HANSON* AND JOHN D. SIMON†‡

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*Communicated by Mostafa A. El-Sayed, Georgia Institute of Technology, Atlanta, GA, July 6, 1998 (received for review March 16, 1998)*

ABSTRACT **The premature photoaging of the skin is mediated by the sensitization of reactive oxygen species after absorption of ultraviolet radiation by endogenous chromophores. Yet identification of UV-A-absorbing chromophores in the skin that quantitatively account for the action spectra of the physiological responses of photoaging has remained elusive. This paper reports that the *in vitro* action spectrum for singlet oxygen generation after excitation of *trans*-urocanic acid mimics the *in vivo* UV-A action spectrum for the photosagging of mouse skin. The data presented provide evidence suggesting that the UV-A excitation of *trans*-urocanic acid initiates chemical processes that result in the photoaging of skin.**

Most of the visible signs of aging result from chronic exposure of the skin to ultraviolet radiation (1–2). Unlike chronologically aged skin that results from a general atrophy and a gradual decline in the production of the dermal matrix (3), UV-A (320–400 nm) photoaged skin is characterized by a gross increase in the elastic fibers (elastin, fibrillin, and desmosine) of the skin replacing the collagenated dermal matrix (elastosis) (4–5), an increase in glycosaminoglycans (4–5), collagen cross-linking, epidermal thickening (4–6), and an increase in the number of dermal cysts (7). The deep lines, leathered appearance, and the sagging of the skin surface typically associated with "old age" are thought to result from UV-induced photodamage to the skin and to occur over the course of a lifetime (3). Although obvious differences between photoaged and chronologically aged skin exist, the anatomic basis of the visible signs of photoaging is not understood fully (4, 8). Absorption of UV-A must induce photobiologic effects within the skin that lead to the visible and histological differences of photoaged skin, and, although the mechanisms by which UV-A-induced photodamage occur have not been completely determined, reactive oxygen species are postulated to play a role (9). The natural shift toward a more prooxidant state in chronologically aged skin then could be exacerbated by absorption of UV-A radiation by endogenous chromophores like NADH/NADPH (10–14), tryptophan (15), and riboflavin (9), which then sensitize the formation of reactive oxygen species. Solar radiation has been shown both to reduce the antioxidant population in the skin (16) and to sensitize the production of reactive oxygen species such as singlet oxygen, hydrogen peroxide, and the superoxide anion (9–19), increasing the potential for reactions like the oxidation of lipids and proteins (17) that influence the degree of cross-linking between collagen and other proteins (9) within the skin.

The first step toward identifying the chromophore(s) responsible for physiological changes, such as those seen in photoaged skin, is to match the action spectrum for the physiological change with the absorption spectrum of the chromophore (20). Once such a comparison is made, the pathways that lead to the physiological change can be unraveled. For example, the epidermal chromophore *trans*-urocanic acid (*t*-UA) has received considerable attention in the last 15 years, following the discovery that its broad and structureless absorption spectrum from 260 nm to 320 nm mimicked that of the immune suppression action spectrum of contact hypersensitivity (21). Now, a direct relationship between UV absorption by *t*-UA and UV-induced immunomodulation has been postulated (21), and the mechanism by which *t*-UA mediates these physiological events is a topic of current research (22–29). A similar quantitative comparison between photoaging action spectra and the absorption spectra of endogenous chromophores in the UV-A has remained elusive.

In this report, results from pulsed laser photoacoustic spectroscopy (30, 31) are presented. This technique allows one to study the energetics and dynamics of nonradiative processes. These processes can be photophysical (e.g., internal conversion, intersystem crossing) or photochemical (e.g., isomerization, electron transfer) in nature. The underlying principle of the technique is that a nonradiative decay generates a thermal acoustic wave in the sample, which is detected by a piezoelectric transducer. The experiment measures dynamic processes that occur within a certain time window. This time window is determined by the sample cuvette design and the frequency response of the detection transducer. The sample cuvette is calibrated so that the observed signal can be quantified in terms of the fraction of the photon energy that is released by the sample as heat on the timescale of the transducer response. In addition, kinetic information can be extracted from the differences in the temporal properties of the waveforms for the sample and reference compound. In the present study, excitation in the UV-A is achieved by using either a picosecond or femtosecond laser system.

In this report, we focus on the UV-A absorption of *t*-UA between 310 nm and 351 nm and the potential role of *t*-UA in the photoaging of the skin. Examination of Fig. 1 shows that this region corresponds to the tail of the absorption spectrum. Irradiation throughout this spectral region has been shown recently to induce photoisomerization of *t*-UA to its *cis* isomer (*c*-UA) (32–34). Excitation of a pH 7.2 solution of *t*-UA at 310 nm results in photoisomerization in which the quantum yield for the conversion of *t*-UA to *c*-UA at this wavelength is ≈0.49 (35, 36). Previous pulsed laser photoacoustic spectroscopic results revealed that essentially all of the incident photon energy is released as heat on the subnanosecond time-scale at this excitation wavelength (37). This result is consistent with time-resolved absorption studies that indicate that the photoisomerization reaction takes place within the singlet manifold of electronic states on a picosecond timescale (38). Intersystem crossing from the initially populated excited singlet state to the triplet-state manifold does not compete with isomerization to *c*-UA after irradiation at 310 nm (37, 38). In the present report, pulsed laser photoacoustic spectroscopy (37) is used to study the UV-A portion of the *t*-UA

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Abbreviation: *t*-UA, *trans*-urocanic acid.

‡To whom reprint requests should be addressed.

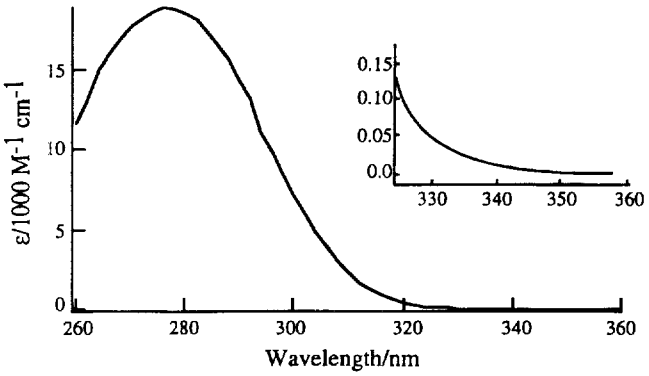


FIG. 1. The absorption spectrum of naturally occurring *trans*-urocanic acid, pH 7.2 (solid line). The broad and structureless spectrum masks the complicated wavelength-dependent photochemistry exhibited by the chromophore. Wavelength-dependent isomerization between *t*-UA and photoinduced *cis*-urocanic acid results from the presence of weakly coupled distinct electronic states between 266 and 400 nm (35–37). In addition, as shown in this report, there is a weak absorption band of *t*-UA from 320 to 360 nm. This region of the spectrum is enlarged in the inset.

absorption spectrum from 310 nm to 351 nm. We find that the photoacoustic signal in this region of the spectrum is wavelength-dependent. Starting at 320 nm, the amount of energy retained by the molecule increases with increasing excitation wavelength. The energy retained reaches a maximum at ≈340 nm and then decreases with increasing excitation wavelength. Because of the low extinction coefficient for *t*-UA for wavelengths >370 nm, the photoacoustic technique could not be used to determine whether energy is retained by *t*-UA after excitation in this region. Recent studies establish that photoisomerization occurs after excitation of *t*-UA in the UV-A (320–400 nm) (32–34). The data obtained in the present study reveal that the energy retained after excitation at 340 nm is almost three times the energy difference between the two isomers of UA [110 kJ·mol$^{-1}$ vs. 40 kJ·mol$^{-1}$ (37)]. Consequently, we can conclude that photoisomerization is not the sole photochemical process that is initiated by UV-A exposure. To account for this observed energy storage, a long-lived intermediate must be formed. The absence of any time delays between the sample and reference photoacoustic waves indicate that this intermediate is formed on the subnanosecond timescale and that its lifetime is longer than the instrument response time, hundreds of nanoseconds (37). Therefore, its decay kinetics do not contribute to the photoacoustic signal. The absence of any photochemistry except isomerization contributing to the photoacoustic signal requires that this intermediate be a long-lived excited state of the molecule, and so it is reasonable to conclude that this state is an excited triplet state of *t*-UA, *vide infra*. Because excitation at 310 nm accesses an electronic state that only leads to isomerization (37–39), it is also reasonable to conclude that excitation between 320 nm and 351 nm populates two distinct but overlapping excited electronic states: the same state that leads to isomerization at 310 nm and an electronic state that either directly or by intersystem crossing populates the long-lived excited triplet state. As a result, the wavelength-dependent photoacoustic measurements determine the action spectrum for triplet state formation for excitation of *t*-UA in the UV-A. *t*-UA does not exhibit any phosphorescence in room temperature solutions. As a result, conventional optical spectroscopic techniques, e.g., collection of a phosphorescence excitation spectrum, cannot be used to determine the line shape of action spectrum for triplet formation. Fig. 2*A* shows this action spectrum as determined from these photoacoustic measurements. This action spectrum is in excellent agreement with the action spectrum for photoinduced sagging of mouse skin (Fig. 2*B*; ref. 40).

Given the agreement between the two action spectra shown in Fig. 2, we undertook *in vitro* experiments to elucidate the

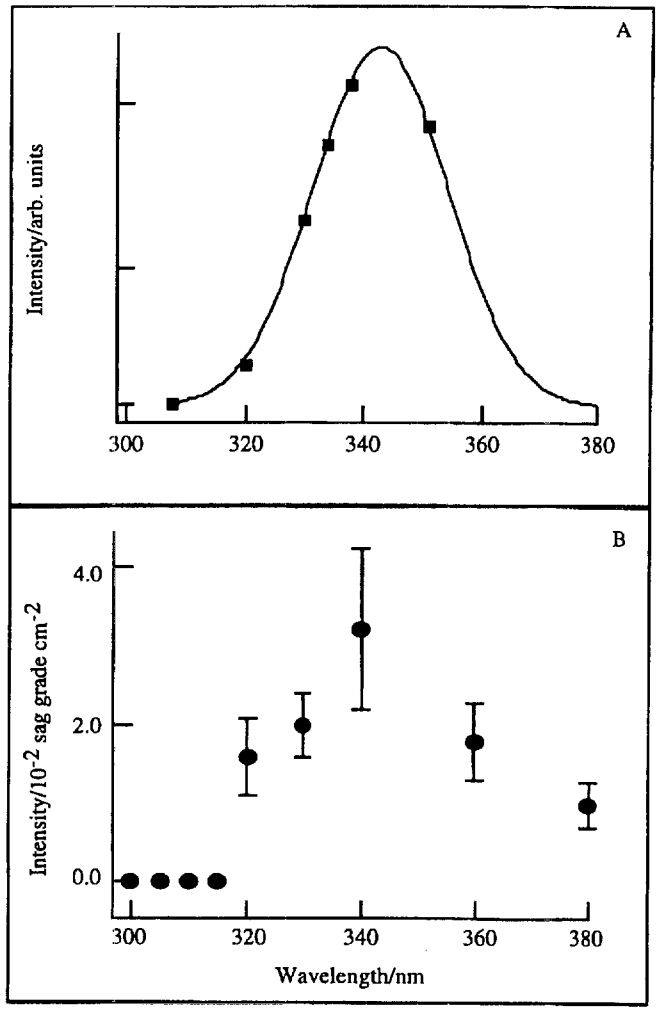


FIG. 2. (*A*) The line shape of action spectrum for triplet formation (and singlet oxygen generation) for *trans*-urocanic acid in a deoxygenated pH 7.2 solution (solid line). The line-shape was determined by fitting a Gaussian line-shape function to the photoacoustic data collected from 320 to 360 nm (data points). Each irradiation wavelength was generated by using a home-built temperature-tuned optical parametric amplifier pumped by a home-built Nd:YLF (1 kHz, 1.054 $\mu$) regenerative amplifier. The $10^{-3}$ M *t*-UA samples had an optical density of between 0.05 and 0.1, and, at each irradiation wavelength, the *t*-UA optical density was matched to within 3% of the optical density of the standard bromocresol purple. (*B*) The measured *in vivo* action spectrum for the photosagging of mouse skin taken from the results of Bissett and coworkers (reprinted with permission from ref. 40). The *in vivo* action spectrum mimics the action spectrum shown in *A*.

photochemical event that mediates this physiological response. Specifically, pulsed laser photoacoustic data after UV-A excitation of *t*-UA were collected both in deoxygenated and oxygen-saturated solutions. In the presence of oxygen, the identical line shape as that reported in Fig. 2*A* is observed. However, when compared with the deoxygenated sample, the oxygen-saturated solution retained significantly less heat. These data showed that oxygen quenches the long-lived state of *t*-UA generated from UV-A irradiation. Quenching reactions of triplet states of organic molecules by $O_2$ are common, and energy transfer from an excited triplet state to $O_2$ leads to the formation of $O_2(^1\Delta_g)$ (41). We have confirmed that $O_2(^1\Delta_g)$ is generated after the UV-A excitation of *t*-UA at 351 nm by measuring the subsequent $^1\Delta_g \rightarrow {}^3\Sigma_g$ emission of $O_2$. This result confirms the above assignment that the long-lived excited state of *t*-UA that is formed on UV-A irradiation is an excited triplet state of the molecule. From the similarity of the

action spectra shown in Fig. 2, we propose that the generation of $O_2(^1\Delta_g)$ that results from the UV-A excitation of *t*-UA initiates chemical processes that lead to the physiological responses characteristic of UV-A photoaged skin. It needs to be noted that, once a photostationary state is achieved, the concentration of *c*-UA exceeds that of *t*-UA in the skin. For example, the photostationary state for photoexcitation at 313 nm is 34% *t*-UA (36). Thus, the reactions of *c*-UA also could contribute to the action spectra of urocanic acid *in vivo*. Whether *c*-UA generates $O_2(^1\Delta_g)$ after UV-A excitation is not known. Work is currently in progress to quantify the photoreactions of *c*-UA *in vitro*.

It is important to emphasize that we are not comparing the *in vivo* action spectrum of the photosagging of skin with the overall absorption spectrum of *t*-UA. Rather, the *in vivo* action spectrum is being compared with a weak transition that contributes to the photochemically complicated, yet structureless absorption spectrum of *trans*-urocanic acid. At first glance, the *t*-UA absorption spectrum would appear to be completely unrelated to any physiological response in the UV-A. However, the present work shows that one must consider the possible role of weak transitions of endogenous absorbing chromophores in initiating physiological responses. This includes chromophores like NADH/NADPH, tryptophan, and riboflavin that have been postulated to play a role in photoaging (18, 19, 42). These chromophores have been implicated in photoaging, but a quantitative comparison between their absorption spectra and photoaging action spectra has not been made. Additional action spectra (skin fold thickening, glycosaminoglycan production, collagen damage, cellularity increase, and elastosis in mice models) also reflect a UV-A component that have a similar line shape as the triplet state action spectrum of *t*-UA (40). It would not be surprising if the generation of $O_2(^1\Delta_g)$ by energy transfer from the excited triplet state of *t*-UA in the UV-A also contributes to these physiological responses.

$O_2(^1\Delta_g)$ is not a "selective" reactant, and it initiates a wide range of physiological responses. Recent reports of the UV-A irradiation of fibroblasts indicate that singlet oxygen is both an early intermediate in the signaling pathway of interstitial colleganase induction, preceding the synthesis of the proinflammatory cytokines interleukin 1 and interleukin 6 (43), and can activate JNKs (stress-activated kinases), which can affect gene expression (44). Correlation between UV-A and singlet oxygen also have been discussed for the induced synthesis of mRNA heme oxygenase-1 (45, 46), colleganase (43, 47, 48), and intercellular adhesion molecule-1 (49). Whether *t*-UA is a major contributing source of the singlet oxygen that causes these responses requires more research.

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1. Warren, R., Gartstein, V., Kligman, A. M., Montagna, W., Allendorf, R. A. & Ridder, G. M. (1991) *J. Am. Acad. Dermatol.* **25,** 751–760.
2. Frances, C. & Robert, L. (1984) *Int. J. Dermatol.* **23,** 166–179.
3. Bernstein, E. F. & Uitto, J. (1996) *Clin. Dermatol.* **14,** 143–151.
4. Kligman, L. H. (1996) *Clin. Dermatol.* **14,** 183–195.
5. Kligman, L. H., Akin, F. J. & Kligman, A. M. (1986) *J. Invest. Dermatol.* **84,** 272–276.
6. Kligman, L. H., Sayre, R. M. & Kaidby, K. H. (1987) *Photochem. Photobiol.* **45,** Suppl., 7s.
7. Bissett, D. L., Hannon, D. P. & Orr, T. V. (1987) *Photochem. Photobiol.* **46,** 367–378.
8. Kligman, A. M., Zheng, P. & Lavker, R. M. (1985) *Br. J. Dermatol.* **113,** 37- 42.
9. Pathak, M. A. & Carbonare, M. D. (1992) in *Biological Responses to Ultraviolet A Radiation*, ed. Urbach, F. (Valdenmar, Overland Park, Kansas), pp. 189–208.
10. Sohal, R. S. & Weindruch, R. (1996) *Science* **273,** 59–63.
11. Noy, N., Schwartz, H. & Gafni, A. (1985) *Mech. Ageing Dev.* **29,** 63–69.
12. Sohal, R. S. (1989) in *Advances in Myochemistry*, ed. Benzi, G. (John Libbey, Paris), pp. 112–121.
13. Czochralska, B., Bartosz, W., Shugar, G. & Shugar, D. (1984) *Biochim. Biophys. Acta.* **801,** 403–409.
14. Cunningham, M. L., Johnson, J. S., Giovanazzi, S. M. & Peak M. J. (1985) *Photochem. Photobiol.* **42,** 125–128.
15. McCormick, J. P., Fisher, J. R., Pachlatko, J. P. & Eisenstark, A. (1976) *Science* **191,** 468–469.
16. Shindo, Y., Witt, E. & Packer, L. (1993) *J. Invest. Dermatol.* **100,** 260–265.
17. Vile, G. F. & Tyrrell, R. M. (1995) *Free Radical Biol. Med.* **18,** 721–730.
18. Peak, M. J. & Peak, J. G. (1986) in *The Biological Effects of UVA Radiation,* eds. Urbach, F. & Gange, R. W. (Praeger, New York), pp. 42–52.
19. Peak, M. J. & Peak, J. G. (1989) *Photodermatology* **6,** 1–15.
20. Lea, D. E. (1947) *Actions of Radiations on Living Cells* (Macmillan, New York).
21. De Fabo, E. C. & Noonan, F. P. (1983) *J. Exp. Med.* **157,** 84–98.
22. Kurimoto, I. & Streilein, J. W. (1992) *J. Invest. Dermatol.* **99,** 69s–70s.
23. Kurimoto, I. & Streilein, J. W. (1992) *J. Immunol.* **148,** 3072–3078.
24. Moodycliffe, A. M., Kimber, I. & Norval, M. (1992) *Immunology* **77,** 394–399.
25. Palasynski, E. W., Noonan, F. P. & De Fabo, E. C. (1991) *Photochem. Photobiol.* **55,** 165–171.
26. Uksila, J., Laihia, J. K. & Jansen, C. T. (1994) *Exp. Dermatol.* **3,** 61–65.
27. Norval, M., Simpson, T. J., Bardshiri, E. & Howie, S. E. M. (1989) *Photochem. Photobiol.* **49,** 633–639.
28. Gilmour, J. W., Norval, M., Simpson, T. J., Neuvonen, K. & Pasenen, P. (1993) *Photodermatol. Photoimmunol. Photomed.* **9,** 250–259.
29. Cosmetic Ingredient Review Expert Panel (1995) *J. Am. Coll. Toxicol.* **14,** 386–421.
30. Peters, K. S. & Snyder, G. J. (1988) *Science* **241,** 1053–1057.
31. Braslavsky, S. E. & Heibel, G. E. (1991) *Chem. Rev. (Washington, D.C.)* **92,** 1381–1410.
32. De Fabo, E. C., Reilly, D. C. & Noonan, F. P. (1992) in *Biological Responses to Ultraviolet A Radiation*, ed. Urbach, F. (Valdenmar, Overland Park, Kansas), pp. 227–238.
33. Kammeyer, A., Teunissen, M. B. M., Pavel, S., De Rie, M. A. & Bos, J. D. (1995) *Br. J. Dermatol.* **132,** 884–891.
34. Webber, L. J., Whang, E. & De Fabo, E. C. (1997) *Photochem. Photobiol.* **66,** 484–492.
35. Morrison, H., Bernasconi, C. & Pandey, G. (1984) *Photochem. Photobiol.* **40,** 549–550.
36. Morrison, H., Avnir, D., Bernasconi, C. & Fagan, G. (1980) *Photochem. Photobiol.* **32,** 711–714.
37. Hanson, K. M., Li, B. & Simon, J. D. (1997) *J. Am. Chem. Soc.* **119,** 2715–2721.
38. Li, B., Hanson, K. M. & Simon, J. D. (1997) *J. Phys. Chem.* **101,** 969–972.
39. Hanson, K. M. & Simon, J. D. (1998) *Photochem. Photobiol.* **67,** 538–540.
40. Bissett, D. L., Hannon, D. P. & Orr, T. V. (1989) *Photochem. Photobiol.* **57,** 763–769.
41. Gilbert, A. & Baggott, J. (1991) *Essentials of Molecular Photochemistry* (CRC, Boca Raton, FL).
42. Pathak, M. A. (1989) *Photochem. Photobiol.* **49,** 3s–4s.
43. Wlaschek, M., Wenk, J., Brenneisen, P., Briviba, K., Schwarz, A., Sies, H. & Scharfetter-Kochanek, K. (1997) *FEBS Lett.* **413,** 239–242.
44. Klotz, L. O., Briviba, K. & Sies, H. (1997) *FEBS Lett.* **408,** 289–291.
45. Basu-Modak, S. & Tyrell, R. M. (1993) *Cancer Res.* **53,** 4505–4510.
46. Vile, G. F. & Tyrell, R. M. (1993) *J. Biol. Chem.* **268,** 14678–14681.
47. Scharfetter-Kochanek, K., Wlaschek, M., Briviba, K. & Sies, H. (1993) *FEBS Lett.* **331,** 304–306.
48. Wlaschek, M., Briviba, K., Stricklin, G. P., Sies, H. & Scharfetter-Kochanek, K. (1995) *J. Invest. Dermatol.* **104,** 194–198.
49. Grether-Beck, S., Olaizola-Horn, S., Schmitt, H., Grewe, M., Jahnke, A., Johnson, J. P., Briviba, K., Sies, H. & Krutmann, J. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 14586–14591.